TECHNICAL MANUSCRIPT 121

AMINO ACID COMPOSITION OF UREDOSPORES OF PUCCINIA GRAMINIS VAR. TRITICI

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AMINO ACID COMPOSITION OF UREDOSPORES OF PUCCINIA GRAMINIS VAR. TRITICI

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ABSTRACT

Uredospores of Puccinia graminis var. tritici (Race 56) were analyzed quantitatively for total free amino acids and ninhydrin-positive substances by ion-exchange chromatography. Extracts of these substances were obtained by leaching the spores and by re-extracting leached spores with boiling water. Thirty-five ninhydrin-positive compounds were found and identified. The leach extract differed quantitatively from the boiled extract although both contained the same 35 substances. It is proposed that the spores contain ninhydrin-positive substances coating the spore wall that are easily extracted and other ninhydrin-positive substances in the protoplasm that can be extracted only with difficulty.

I. INTRODUCTION

Interest in the amino acid content of rust uredospores has developed in conjunction with studies of self-inhibition of uredospore germination.[1,2] It has also been proposed that races of wheat rust could be differentiated by their characteristic amino acid content.[3] Additionally, the amino acid content of rusted and nonrusted wheat plants has been compared to assess the influence of various factors in rust development.[4]

Previously reported analyses of the amino acid composition of wheat rust uredospores[3,5] appear to differ because of the conditions of sampling and analysis. Consequently, the present investigation was undertaken (a) to provide a more accurate amino acid analysis of uredospores of one race of stem rust and (b) to determine if differences exist between the amino acid content of the interior and the outer surface of the spores.

II. MATERIALS AND METHODS

Uredospores of Puccinia graminis var. tritici (Eriks. & Henn.) Guyot, Race 56, were produced in a greenhouse controlled at 21° ± 2°C on seedling plants of Baart wheat (C.I. 1697). Fifteen to 20 seedlings per four-inch clay pot were grown in loamy potting soil fumigated with methyl bromide several days prior to use. Pots were held in galvanized iron trays and watered from the bottom as needed.

Thirteen days after seeding, all plants were dusted with uredospores of Race 56 and held overnight in a moisture-saturated atmosphere at 20°C to permit infection. Ten to 20 pustules per leaf, uniformly distributed, resulted from the inoculation. Fourteen days after inoculation the test spores were removed from the host plants with cyclone collecting devices. Spores were stored in screw-capped vials at 4°C until used.

A sample of freshly harvested spores was desiccated in vacuo over P_2O_5 to obtain the spore dry weight. Another undried one-gram sample was weighed accurately and extracted with 20 milliliters of water by shaking vigorously for five minutes. The mixture was centrifuged and the subnatant extract withdrawn. The extraction was repeated until a drop of the solution no longer gave a positive test when spotted on filter paper and sprayed with ninhydrin. Four extractions removed all of the soluble amino acids. These extracts from the "coating" were combined, lyophilized, and stored in vacuo over P_2O_5 until analyzed.

The extracted spores were filtered, washed with acetone, defatted overnight in a Soxhlet apparatus with ether, and air-dried. An accurately weighed sample was refluxed with boiling water (20 ml per gram) for one hour, filtered, washed, and the washings pooled. It was necessary to deproteinize the boiled extract (hereafter referred to as the "protoplasm" extract) with one per cent picric acid (three volumes per volume of extract). The "coat" extract obtained by shaking the spores with water did not contain any material precipitable by picric acid and was considered protein-free. The deproteinized extract was freed of excess picric acid by passage through Dowex 2-X10 ion exchange resin according to the method of Stein and Moore.[6] Deproteinized samples were lyophilized and stored as described above. The amino acid analyses were performed with a Phoenix automatic amino acid analyzer and are accurate to plus or minus three per cent. The program developed by Spackman, Stein, and Moore[7] was employed for the analysis. As glutamine, asparagine, and glutathione cannot be resolved satisfactorily by these procedures, the programming was modified to determine these substances. Thus, a sample of spore extract was analyzed on a 150-cm column of Amberlite 1R-120 resin, using the recommended 30° to 50°C temperature system of Spackman et al. In the resulting chromatogram, asparagine and glutamine emerge together, with oxidation products of glutathione appearing as dispersed peaks. Consequently, an identical spore-extract sample was hydrolyzed (6 N HCl, 24 hours, 110°C) in vacuo in a sealed ampoule to convert glutamine to glutamic acid and asparagine to aspartic acid. During this hydrolysis, glutathione and its derivatives were hydrolyzed to glycine, glutamic acid, and cystine or one or more of its oxidized forms. The acid was removed from the hydrolyzate by lyophilization, and the residue was analyzed to determine the increase in glutamic acid, glycine, and aspartic acid above that of the unhydrolyzed control. The difference between glycine in the hydrolyzed and unhydrolyzed samples was assumed to represent the glutathione value, and was also equal to the number of moles of glutamic acid resulting from the glutathione hydrolysis. The amount of glutamic acid in the unhydrolyzed sample plus that resulting from glutathione hydrolysis was then subtracted from the amount of glutamic acid in the hydrolyzed sample to give the amount of glutamine present originally. Similarly, the asparagine content was the difference between the aspartic acid content of the hydrolyzed and unhydrolyzed extracts.

Amino acids and other ninhydrin-positive substances were identified by their position of emergence on the chromatogram and by the characteristic ratio of the areas of their integrated elution peaks at 570 and 440 millimicrons.

Experimental values were expressed as micromoles of amino acids per gram of dry spores to allow comparisons on a molecular basis. In addition, the values were converted into absolute amounts on a basis of micrograms per gram of spores so that the weight of each constituent could be expressed as a percentage of the total amount in order to determine if our results could be compared with previously published work.

III. RESULTS

The extract of the coating constituted 21.6 per cent; the protoplasm extract, 5.12 per cent of the dry weight of the spores. Consequently, the spores contain approximately 26 per cent or more extractable solids if the soluble protein of the protoplasm is considered. The coating contained 0.57 per cent and the spore protoplasm 0.63 per cent ninhydrin-positive materials. Thus, approximately 1.2 per cent of the spore weight was composed of free amino acids.

Table I shows the amino acid content of the uredospores. Twenty-three ninhydrin-positive substances were present in quantities greater than trace amounts. Twelve substances were detected in traces. On a weight basis, the chief constituents in the extract of the coating were glutamine, asparagine, glutathione, and alanine, but on a molecular basis glutamine, ammonia, alanine, and asparagine predominated. The protoplasm composition showed glutamine, glutathione, glutamic acid, and ammonia as the chief constituents by weight and, on a molecular basis, the same four substances predominated as in the coating.

Comparison of the coating and the protoplasm compositions shows that the relative molecular ratios of many amino acids are different in each component, and in some cases a component may predominate in the protoplasm but not in the coating. Thus, the coating shows a molecular ratio of aspartic acid to threonine of 1.2:0.4 and the protoplasm shows 0.92:0.98 as the ratio for these substances. Similarly, the molecular ratios of asparagine to glutamic acid, aspartic acid to serine, and serine to glutathione are different and inverse.

Major quantities of conjugated amino acids were not present in the analyses. The only increase in concentration of any particular acids after hydrolysis, other than the glutamine-glutamic acid and asparagine-aspartic acid transformations, was observed in glycine and cystine, and was attributed to hydrolysis of glutathione or its oxidation products.

TABLE I. ABSOLUTE AND RELATIVE AMOUNTS OF FREE AMINO ACIDS AND NINHYDRIN-POSITIVE SUBSTANCES IN SPORES OF PUCCINIA GRAMINIS VAR. TRITICI RACE 56

	Coat Extract			Protoplasm Extract		
Substance	µmole/gm	µgm/gm	% of Total	µmole/gm	µgm/gm	% of Total
Glutathione	1.96	602	10.5	1.70	522	8.3
Aspartic acid	1.16	154	2.7	0.92	122	2.0
Threonine	0.39	46.4	0.8	0.98	117	1.9
Serine	0.79	83.0	1.5	1.92	202	3.2
Asparagine	4.80	634	11.1	2.37	313	5.0
Glutamine	9.74	1424	24.9	16.30	2383	38.0
Glutamic acid	1.06	156	2.7	2.63	387	6.2
Glycine	0.65	48.8	0.8	1.77	133	2.1
Alanine	5.37	479	8.4	4.56	406	6.5
Valine	2.88	338	5.9	1.20	141	2.2
Cystine/2	1.78	214	3.7	0.58	69.7	1.1
Methionine	0.87	130	2.3	0.44	65.6	1.0
Isoleucine	1.60	210	3.7	0.60	78.7	1.2
Leucine	2.37	311	5.4	0.68	89.2	1.4
Tyrosine	1.00	181	3.2	0.58	105	1.7
Phenylalanine	2.21	365	6.4	0.73	121	1.9
Galactosamine	0.12	21.4	0.4	Trace	-	-
γ-Amino-butyric acid	0.63	65.0	1.1	1.76	181	2.9
Ornithine	0.10	13.2	0.2	0.14	18.5	0.3
Ethanolamine	1.0	61.1	1.1	2.5	153	2.4
Ammonia	5.6	95.2	1.7	22.0	374	6.0
Histidine	0.29	45.0	0.8	0.98	152	2.4
Arginine	0.23	40.0	0.7	0.80	139	2.2
Lysine[a/]	Trace	-	-	Trace	-	-

a. Also detected in trace amounts: proline, glucosamine, α-amino-n-butyric acid, l-methyl histidine, homocystine, β-alanine, α-aminoadipic acid, tryptophane, hydroxylysine, taurine, lanthionine.

IV. DISCUSSION

Two analyses of the amino acid content of Race 56, P. graminis var. tritici, have been reported. Broyles[6] reported the presence of 30 ninhydrin-positive substances and could identify 21; McKillican[3] identified and quantitated 19 amino acids. Neither of these workers reported a differential analysis between spore coating and spore protoplasm, but analyzed the entire spore. The former reported that cystine, asparagine, threonine, homocystine, and glutamic acid were present in his extracts. The latter reported methionine sulfoxides, cysteic acid, no glutamic acid or threonine, did not determine asparagine or glutamine, and was unable to detect homocystine. We confirm Broyles's results except for the presence of lysine, which we detected only in trace amounts. McKillican's results are difficult to reconcile with those of Broyles and with ours unless his experimental protocols are examined critically. In his spore extracts, prepared by refluxing the spores with water, some glutamine and asparagine undoubtedly hydrolyzed to the constituent acids and ammonia, but no glutamic acid was reported. In addition, a small amount of cysteic acid might be expected to form from air oxidation of cysteine or hydrolysis of glutathione followed by oxidation. We think, however, that he may have reported his oxidized glutathione peak as cysteic acid, because both appear within a few effluent volumes of each other in column chromatography. The failure to detect glutamic acid or threonine, however, is inexplicable. Also, his finding that Race 56 contains 41.3 per cent alanine seems unreasonable; this high value probably inadvertently includes the missing glutamine and asparagine in the alanine peak, perhaps because of poor resolution on his chromatographic column. McKillican also reported the sum of the free and conjugated amino acids comprised 11 per cent of the spore weight. This high value may have resulted from incomplete deproteinization of the spore extract. These considerations preclude a fruitful comparison of his tabulated results with ours.

Both workers reported lysine as a spore constituent, but we found this substance present only in trace amounts. We report the presence of ornithine instead, and reconcile our results with theirs by differences in procedure. Both workers identified lysine by paper chromatography. The systems employed by both Broyles and McKillican are incapable of distinguishing between ornithine and lysine. Thus, the cited references[8] of McKillican give R_f values of 0.81 and 0.79 respectively for lysine and ornithine in aqueous phenol, 0.11 each for these substances in collidine-lutidine, and 0.14 and 0.15 in butanol-acetic acid-water. In our systems, using pH 4.26, 0.38 M citrate buffer at 30°C, lysine emerges well after ornithine.

In addition, we report substantial amounts of glutathione. This material emerges partially as oxidized forms, predominantly as a broad band in the proline region. Because proline was present only in trace amounts, the glutathione band did not interfere with the chromatography. A standard procedure used to determine this material is to convert it to the S-sulfonate by

reaction with sulfite.[9] This was inapplicable in our case as the conversion proceeds only from unoxidized glutathione, and appreciable quantities of oxidized forms occur in spores. Consequently, the glutathione was estimated as described previously.

The behavior of the spores when subjected to extraction suggests that they possess a water-soluble coating on the spore wall. It is seen that after leaching exhaustively, the spores still contained more of certain amino acids than had been removed, and that more than half of the amino acids could be obtained only by harsher extraction methods. These findings, coupled with the inconstant inverse ratios of amino acids in leach extracts and boiling water extracts suggests two different sources of amino acids.

This difference probably results from contamination of the spore coat by amino acids of the wheat tissues on which the spores were produced. A comparison of the amino acid content of the spore coat and that of the wheat plant, as reported by McKillican, would not unequivocally settle this point, however. Shaw[4] studied the amino acid composition of wheat leaves infected with stem rust; he reported that the concentration of free amino acids rose steadily until it became more than fourfold that of noninfected leaves at nine days after inoculation, and that maximum concentrations of the different amino acids were reached at different times, causing a progressive change in the free amino acid pool. It would be expected that, under these conditions, uredospores would be in contact with a medium of inconstant composition, and that this would be reflected in the composition of the spore coating.

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